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HIGHLY REACTIVE CONDENSING AGENTS FOR THE SYNTHESIS OF OLIGONUCLEOTIDES BY THE PHOSPHOTRIESTER APPROACH†

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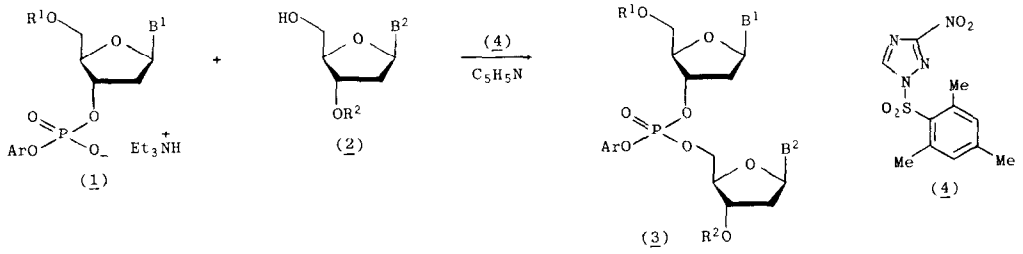
Summary: 4,6-Dinitro-1-(mesitylene-2-sulphonyloxy)-benzotriazole [DMBT, (6b)], 1-(mesitylene-2-sulphonyloxy)-4-nitro-6-trifluoromethylbenzotriazole (6a) and 1-(mesitylene-2-sulphonyl)-4-nitro-1,2,3-triazole [iso-MSNT, (5)] are more reactive condensing agents in the phosphotriester approach to oligonucleotide synthesis than 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole [MSNT, (4)] by factors of at least 10, ca. 4 and ca. 1.3, respectively.

In recent years, the phosphotriester approach has been widely used in the synthesis of oligodeoxyribonucleotides both in solution[1] and on solid supports[2]. The crucial chain lengthening step in this approach (Scheme 1) involves the reaction between a 3'-phosphodiester component (1), a component with a 5'-hydroxy function (2) and a condensing agent in pyridine solution. The condensing agent that appears to be used most widely for this purpose is 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole[3,4] [MSNT, (4)]. The latter reagent is a stable crystalline solid which promotes fairly rapid condensation reactions; it has an additional advantage in that, although it reacts[5] with unprotected uracil (and, to a much lesser extent, thymine) and 2-N-acylguanine residues, the ensuing base modification processes are reversed[5] in the course of a standard unblocking step[3,6] that is carried out towards the end of the synthesis.

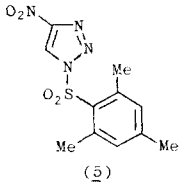
Scheme 1



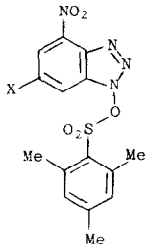
a; R^1 = 9-phenylxanthen-9-yl, R^2 = acetyl, B^1 = B^2 = thymin-1-yl, Ar = 2-chlorophenyl

In solid phase oligonucleotide synthesis, it is desirable that the chain lengthening steps should proceed rapidly and, if possible, quantitatively. The alternative phosphite triester approach[7] involves the use of protected nucleoside 3'-phosphoramidites[8] and an activating agent [such as 1-H-tetrazole], instead of protected nucleoside 3'-aryl phosphates (1) and a condensing agent [such as MSNT (4)]; the phosphite triester approach benefits from the fact[9] that P(III) are generally more reactive than P(V) acylating agents. While the rates of

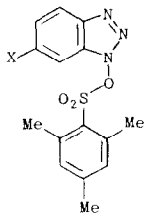
condensation reactions in the phosphotriester approach may be increased by the addition of nucleophilic catalysts[10] or by conducting the reactions above room temperature[11,12], we are unaware of any previous studies concerned with the development of condensing agents that can be used under the same conditions as MSNT (4) [i.e. in anhydrous pyridine solution at room temperature], but which are more reactive. We now report that 1-(mesitylene-2-sulphonyl)-4-nitro-1,2,3-triazole[13] [iso-MSNT, (5)], 1-(mesitylene-2-sulphonyloxy)-4-nitro-6-trifluoromethylbenzotriazole[14] (6a), and especially 4,6-dinitro-1-(mesitylene-2-sulphonyloxy)-benzotriazole[14] [DMBT, (6b)] are all more reactive condensing agents than MSNT (4), and that these reagents can be used under the same reaction conditions as MSNT. On the other hand, 1-(mesitylene-2-sulphonyloxy)-6-nitrobenzotriazole[15] (7b) [Table 1, entry no. 5] is less reactive than MSNT, and the parent compound of this series, 1-(mesitylene-2-sulphonyloxy)-benzotriazole (7a)[16] is extremely unreactive [reaction time under the conditions indicated in Table 1, > 150 hr] as a condensing agent.



(5)



(6) a; X = CF_3
b; X = NO_2



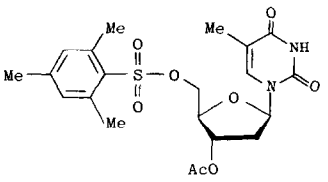
(7) a; X = H
b; X = NO_2

TABLE 1. Condensation Reactions[a] Leading to 5'-O-(9-Phenylxanthen-9-yl)thymidylyl-(3'→5')-(3'-O-acetylthymidine) 2-Chlorophenyl Ester (3a)

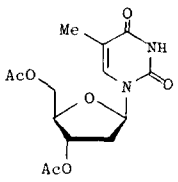
Entry No.	Quantity of (1a) [mmol]	Quantity of (2a) [mmol]	Vol. of Pyridine [ml]	Condensing Agent (Quantity [mmol])	Reaction Time [min]	Isolated Yield of (3a) [%]
1	0.85	0.77	8	(4) (2.32)	20	80
2	0.85	0.77	8	(5) (2.32)	15	76
3	0.85	0.77	8	(6a)(2.30)	5	84
4	0.85	0.77	8	(6b)(2.33)	2	82
5	0.58	0.49	3	(7b)(1.46)	45	73

[a]The nucleotide (1a) and nucleoside (2a) components were dried by evaporation from pyridine [16 ml, except for experiment in entry no. 5 (6 ml)] solution, redissolved in pyridine at room temperature and the condensing agent was then added. After the reaction time indicated, saturated aqueous sodium hydrogen carbonate (2 ml) was added, and the products were worked up and purified by short column chromatography on silica gel. The product (3a) was isolated as a precipitated solid.

Each of the new condensing agents [(5), (6a), (6b) and (7b)] was used in the preparation of 5'-O-(9-phenylxanthen-9-yl)thymidylyl-(3'→5')-(3'-O-acetylthymidine) 2-chlorophenyl ester (3a) [Scheme 1], and their reactivities were compared with the reactivity of MSNT (4) [Table 1]. It can be seen that iso-MSNT (5) [entry no. 2] is only marginally more reactive than MSNT (4) [entry no. 1], but that (6a) and (6b) promoted the condensation reaction [Scheme 1] at least 4 and 10 times, respectively as rapidly as MSNT (4). The comparative reactivity of DMBT (6b) is probably appreciably greater than is suggested by the data in Table 1. Indeed, a t.l.c. examination of the condensation reaction involving (6b) revealed very little, if any, remaining 3'-O-acetylthymidine (2a) after 1 min.



(8)



(9)

As expected, 1-(mesitylene-2-sulphonyloxy)-4-nitro-6-trifluoromethylbenzotriazole (6a) and DMBT (6b) were also found to be more reactive sulphonating agents than MSNT (4). When 3'-O-acetylthymidine (2a) was treated, in separate experiments, with 3.0 molecular equivalents of (4), (6a) and (6b) in pyridine solution under conditions corresponding to those used in the above condensation reactions [Table 1, entries nos. 1, 3 and 4], it was completely converted into its 5'-O-(mesitylene-2-sulphonyl) derivative (8) in 24 hr, 2 hr and 45 min, respectively. The latter compound (8) was not, however, detected as a by-product in any of the condensation reactions described above. Although we favour the protection of the lactam groups of thymine and guanine residues[17] in oligodeoxyribonucleotide synthesis, we treated 0.2M - solutions of 3',5'-di-O-acetylthymidine (9) in anhydrous pyridine with 5 molecular equivalents of (6a) and (6b) in the presence of 0.75 molecular equivalents of diphenyl phosphate[5] at room temperature. In both experiments, ca. 70-75% of the starting material (9) remained after 24 hr.

In conclusion, we believe that of the new reagents described in this paper, DMBT (6b) especially has much potential as a condensing agent in the rapid synthesis of oligonucleotides by the phosphotriester approach both on solid supports and in solution. Indeed, it would seem likely that the use of DMBT (6b) instead of MSNT (4) would considerably narrow the present difference in cycle times[18] between the phosphotriester and phosphite triester approaches.

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REFERENCES AND FOOTNOTES

† This article is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University.

1 For some leading references, see J.B. Chattopadhyaya and C.B. Reese, *Nucleic Acids Res.*, 8, 2039 (1980).

2 M.J. Gait, H.W.D. Mattles, M. Singh, and R.C. Titmas, *J. Chem. Soc., Chem. Commun.*, 37 (1982).

3 C.B. Reese, R.C. Titmas, and L. Yau, *Tetrahedron Lett.*, 2727 (1978).

4 S.S. Jones, B. Rayner, C.B. Reese, A. Ubasawa, and M. Ubasawa, *Tetrahedron*, 36, 3075 (1980).

5 C.B. Reese and A. Ubasawa, *Tetrahedron Lett.*, 21, 2265 (1980).

6 C.B. Reese and L. Zard, *Nucleic Acids Res.*, 9, 4611 (1981).

7 M.D. Matteuchi and M.H. Caruthers, *J. Am. Chem. Soc.*, 103, 3185 (1981).

8 S.L. Beaucage and M.H. Caruthers, *Tetrahedron Lett.*, 22, 1859 (1981).

9 R.L. Letsinger and W.B. Lunsford, *J. Am. Chem. Soc.*, 98, 3655 (1976).

10 V.A. Efimov, A.A. Buryakova, S.V. Reverdatto, O.G. Chakhmakhcheva, and Y.A. Ovchinnikov, *Nucleic Acids Res.*, 11, 8369 (1983); V.A. Efimov, O.G. Chakhmakhcheva, and Y.A. Ovchinnikov, *ibid.*, 13, 3651 (1985).

[11] T.P. Patel, T.A. Millican, C.C. Bose, R.C. Titmas, G.A. Mock, and M.A.W. Eaton, *Nucleic Acids Res.*, 10, 5605 (1982).

[12] S. Ikuta, R. Chattopadhyaya, and R.E. Dickerson, *Nucleic Acids Res.*, 12, 6511 (1984).

[13] 1-(Mesitylene-2-sulphonyl)-4-nitro-1,2,3-triazole (5) was prepared by allowing 4-nitro-1,2,3-triazole [S. Maiorana, D. Pocar, and P.D. Croce, *Tetrahedron Lett.*, 1966, 6043], mesitylene-2-sulphonyl chloride and triethylamine to react together in dichloromethane solution, following the procedure used[4] in the preparation of MSNT (4); it was isolated as a crystalline solid (toluene/cyclohexane), m.p. 88-90°C, in 40% yield. From its ^{1}H n.m.r. spectrum, this material appeared to be a 57:43 mixture of isomers.

[14] Condensing agents [(6a) and (6b)] were prepared from commercially available 4-chloro-3,5-dinitrobenzotrifluoride [Aldrich Chemical Co.] and from 2,4,6-trinitroanisole [O.L. Brady and H.V. Horton, *J. Chem. Soc.*, 127, 2230 (1925)], respectively. The procedure used for the preparation of (6b) is as follows: A solution of hydrazine hydrate (2.0 ml, 41.2 mmol) in absolute ethanol (20 ml) was added dropwise to a solution of 2,4,6-trinitroanisole (10.0g, 41.1 mmol) in ethanol (60 ml) in an atmosphere of nitrogen at 0°C. After 1 hr, the products were concentrated under reduced pressure, and the dark red precipitate was collected by filtration and recrystallized from glacial acetic acid; yield, 7.3g. The latter material (5.0g, 20.6 mmol), hydrazine hydrate (1.0 ml, 20.6 mmol), sodium acetate trihydrate (10.04g, 0.10 mol), glacial acetic acid (4.95 ml, 86.5 mmol) and water (60 ml) were heated together, in an atmosphere of N_2, for 3 hr. Activated charcoal (5g) was then added and the products were filtered, cooled and acidified with 2M - hydrochloric acid (20 ml). 4,6-Dinitro-1-hydroxybenzotriazole [R. Huisgen and V. Weberndörfer, *Chem. Ber.*, 100, 71 (1967)] was collected as a yellow crystalline precipitate; after it had been washed with water (3 × 10 ml) and dried (over P_2O_5, at ca. 0.1 mmHg), it had m.p. 192-194°C dec. (lit. 185-190°C dec.); yield, 2.85g (61%).

M-Aqueous sodium hydroxide was carefully added to a stirred solution of 4,6-dinitro-1-hydroxybenzotriazole (2.0g, 8.88 mmol) in methanol (10 ml) until the pH increased to 7.0 (pH meter). The products were concentrated under reduced pressure, and then dioxane (2 × 10 ml) was added and removed by evaporation. The dried (20°C, 0.1 mmHg) residue (2.0g, 8.09 mmol) was stirred with mesitylene-2-sulphonyl chloride (1.75g, 8.0 mmol) in anhydrous acetonitrile (60 ml) at room temperature for 16 hr. The products were then filtered and the filtrate was evaporated under reduced pressure. Recrystallisation of the residue from cyclohexane-toluene (9:1 v/v) gave 4,6-dinitro-1-(mesitylene-2-sulphonyloxy)-benzotriazole [DMBT, (6b)] as yellow crystals, m.p. 150-151°C dec.; yield, 1.73g (53%); δ_H [$CDCl_3$, 250 MHz] 2.40 (3H, s), 2.53 (6H, s), 7.11 (2H, s), 8.95 (1H, d, J 1.8 Hz), 9.14 (1H, d, J 1.8 Hz).

[15] 1-(Mesitylene-2-sulphonyloxy)-6-nitrobenzotriazole (7b), m.p. 121-123°C, was prepared from 1-hydroxy-6-nitrobenzotriazole [O.L. Brady and J.N.E. Day, J. Chem. Soc., 123, 2258 (1923)], in 62% yield, by the procedure used above[14] for the preparation of (6a) and (6b).

[16] 1-(Mesitylene-2-sulphonyloxy)-benzotriazole (7a), m.p. 115-117°C was prepared, in 52% yield, from 1-hydroxybenzotriazole and mesitylene-2-sulphonyl chloride by the procedure used in the preparation of MSNT (4). Inasmuch as the phosphorylating agent derived from 2-chlorophenyl phosphorodichloridate and 2 mol. equiv. of 1-hydroxybenzotriazole [G. van der Marel, C.A.A. van Boeckel, G. Wille, and J.H. van Boom, *Tetrahedron Lett.*, 22, 3887 (1981)] is a more powerful phosphorylating agent than 2-chlorophenyl phosphorodi-(1,2,4-triazolide)[1], and 1-(mesitylene-2-sulphonyl)-1,2,4-triazole [N. Katagiri, K. Itakura, and S.A. Narang, *J. Chem. Soc., Chem. Commun.*, 325 (1974)] is an effective, albeit relatively unreactive condensing agent, it is surprising that (7a) appears to have so little activity as a condensing agent. The phosphorylating agent derived from 1-hydroxy-6-nitrobenzotriazole and 2,5-dichlorophenyl phosphorodichloridothioate [J.E. Marugg, C. van den Bergh, M. Tromp, G.A. van der Marel, W.J. van Zoest and J.H. van Boom, *Nucleic Acids Res.*, 12, 9095 (1984)] is more reactive than that derived from the latter reagent and 1-hydroxybenzotriazole [Ö. Kemal, C.B. Reese, and H.T. Serafinowska, *J. Chem. Soc., Chem. Commun.*, 591 (1983)].

[17] C.B. Reese and P.A. Skone, *J. Chem. Soc., Perkin Trans. 1*, 1263 (1984).

[18] See 'Oligonucleotide Synthesis. A Practical Approach', M.J. Gait, Ed., IRL, Oxford and Washington, 1984, pp.50, 105.

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